HUNTINGDON LIFE SCIENCES LTD
PO BOX 2
HUNTINGDON
CAMBRIDGESHIRE
PE18 6ES
ENGLAND

TECHNICAL REPORT

Report No.:

Title: **Eye Irritation of in the Rabbit**

Study No.:

External Testing Facility No.:

Test Substance:

Study Director:

Sponsor:

Sponsor Representative:

Testing Facility: **Huntingdon Life Sciences Ltd.**
PO Box 2
Huntingdon
Cambridgeshire
PE18 6ES
ENGLAND

Study Completion Date: **10 February 2000**

Security Statement:

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ABSTRACT

The objective of this study was to assess the eye irritation potential of following a single instillation to the rabbit eye.

The study was conducted using the EPA Health Effects Test Guidelines, OPPTS 870.2400 Acute Eye Irritation EPA 712-C-98-195, August 1998.

was administered at a dose of 0.1 ml into the lower everted lid of one eye of three female rabbits. All rabbits were scored for irritation for four days (approximately 60 minutes and 24, 48 and 72 hours after test substance instillation). Additional observations were made for all three animals four, seven, 14 and 21 days after instillation.

There was no evidence of systemic response to treatment. A single instillation of into the eye of the rabbit elicited dulling of the cornea developing into corneal opacification (Grade 1) in one animal, corneal opacification (Grade 2 or 3) in two animals and vascularisation on the cornea in all three animals. Iridial inflammation (Grade 1), a diffuse beefy red colouration of the conjunctivae, swelling with lids about half-closed and discharge with moistening of the lids and hairs and considerable area around the eyes was seen in all three animals. Corneal opacification (Grade 1) persisted in two animals at study termination 21 days after instillation, together with hyperaemia of the blood vessels of the conjunctivae with or without slight swelling in all three animals.

As a result of the ocular reactions observed, is classified as a severe irritant.

GLP COMPLIANCE STATEMENT

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August, 1989.

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

The UK Good Laboratory Practice Regulations 1997 (Statutory Instrument No 654) and from 14 December 1999, the UK Good Laboratory Practice Regulations 1999 (Statutory Instrument No 3106).

EC Council Directive 87/18/EEC of 18 December 1986 (Official Journal No L 15/29), and from 1 May 1999 EC Commission Directive 1999/11/EC of 8 March 1999 (Official Journal No L 77/8).

The raw data has been reviewed by the Study Director, who certifies that the information contained in this report is consistent with and supported by the raw data.

10 Feb 2000

Date

Study Director,
Huntingdon Life Sciences Ltd.

QUALITY ASSURANCE STATEMENT

Study Title: Eye Irritation of in the Rabbit

Study Number:

Study Director:

This study has been audited by Huntingdon Life Sciences Ltd. Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon.

Inspections were made by the Quality Assurance Department of various phases of the study conducted at Huntingdon and described in this report. The dates on which the inspections were made and the dates on which the findings were reported to the Study Director and to Management, Huntingdon Life Sciences Ltd. are given below.

Date of Inspection	Study Phase	Finding reported to:	
		Study Director	Management
20 October 1998	Protocol review	21 October 1998	21 October 1998
23 October 1998	Husbandry	23 October 1998	23 October 1998
23 October 1998	Weighing of animals	23 October 1998	23 October 1998
23 October 1998	Dose Procedure	23 October 1998	23 October 1998
10 December 1998	Report audit	10 December 1998	10 December 1998

3 February 2000
Date

Quality Assurance Group Manager,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

APPROVAL SIGNATURES

This report consists of Pages 1 through 17 including Table 1 and Appendices 1and 2.

10 Feb 00
Date

Management,
Huntingdon Life Sciences Ltd.

10 Feb 2000
Date

Study Director,
Department of Acute Toxicology,
Huntingdon Life Sciences Ltd.

9 February 2000
Date

Senior Study Supervisor,
Department of Acute Toxicology,
Huntingdon Life Sciences Ltd.

21 Jan. 2000
Date

Sponsor Representative,

STUDY INFORMATION

Study Initiation Date:	October 15, 1998
Experimental Start Date:	October 23, 1998
Experimental Termination Date:	November 25, 1998
Study Completion Date:	10 February 2000
Study Director:	
Sponsor:	
Sponsor Representative:	
Study Supervisor:	
Senior Technician for the study:	
Head, Department of Acute Toxicology:	
Chief Technician:	
Director Quality Practices:	
Head, Department of Analytical Chemistry and Pharmacy:	
Head, Department of Microbiology:	
Head, Veterinary Services:	

Eye Irritation of in the Rabbit

I. INTRODUCTION

The objective of this study was to assess the eye irritation potential of following a single instillation into the rabbit eye.

II. MATERIALS AND METHODS

A. Test Substance : lot number BN028339, was received at Huntingdon Life Sciences Ltd. on April 6, 1998. The test substance was a pale yellow liquid, and was stored at room temperature (ambient temperature between 10 and 30°C). The Huntingdon Test Substance Data Sheet indicated that the test substance was stable until 28 February 2001. The test substance, as received, is regarded as the "pure" material and is representative of All the remaining test substance will be returned to the Sponsor after the completion of all the relevant studies, with the exception of a 1 g sample which will be retained by Huntingdon Life Sciences Ltd. Test substance characterisation has been carried out by the Sponsor (Study number). As the test substance was administered as supplied, assessment of solubility was not applicable in this study. The absorption of the test substance was not quantitated.

B. Dosage Formulation: The test substance was shaken and administered undiluted.

C. Animals: New Zealand White rabbits (HsdPoc:NZW) weighing between 1928 and 2753g at receipt were obtained from Harlan UK Ltd, Shaw's Farm, Blackthorn, Bicester, Oxon, England on October 8, 1998, and kept in isolation. They were observed daily for signs of ill-health and following a review of health monitoring procedures (absence of clinical observations and satisfactory body weight gain) by a veterinary officer, three healthy rabbits were randomly selected from the stock order after 11 days of acclimatisation.. All three rabbits were female. The animals were identified by a numbered aluminium tag placed through the edge of one ear on arrival. These numbers were unique within the Huntingdon Life Sciences Ltd. Acute Toxicology Department throughout the duration of the study. The cage was identified by a coloured label displaying but not limited to the study schedule number, animal number and initials of the Study Director and Home Office licencee. Rabbits of the New Zealand White strain were chosen as the test species as they have been shown to be a suitable model for eye irritation studies and are the species recommended in the test guidelines. The rabbits were dosed by instillation into the eye as the test substance may come into contact with the eye during handling or use.

D. Food and Water: The rabbits were provided, *ad libitum*, with a standard laboratory diet, SDS Stanrab (P) SQC Rabbit Diet (supplier: Special Diet Services Ltd, Witham, Essex) and

drinking water via an automatic watering system (supplier: Anglian Water). Autoclaved hay was supplied three times weekly. The batches of diet were analysed once, by the supplier, for nutrients, possible contaminants and micro-organisms, likely to be present in the diet, and which, if in excess may have an undesirable effect on the test system. Results of routine physical and chemical analyses of drinking water performed by the supplier are made available to Huntingdon Life Sciences Ltd. as quarterly summaries. Water was supplied in conformity with EC Directive 80/778/EEC and UK Water Act 1989 and subsequent amendments. No contaminants capable of adversely affecting the integrity or interpretation of the results from this study were known to be present in the basal diet or the drinking water during the conduct of this study. The Study Director reviewed the feed and water analyses. The certificates of analyses will be lodged in Huntingdon Life Sciences Ltd. Archives. Samples of water were taken from the drinking water at source in the animal room prior to the study start. The samples were analysed for microbial contaminants (total viable count, coliform count and *E.Coli* count) by Huntingdon Life Sciences Ltd. Department of Microbiology. A certificate of analysis is appended to this report.

E. Housing and Environment: The rabbits were housed individually in suspended metal cages with perforated floors measuring 45.5 cm high, 76 cm wide and 60.5 cm deep (floor area 4598 cm^2). The cage size is in compliance with UK animal welfare guidelines. Absorbent cage liners were placed in the pan below the metal mesh floor of the animal cage to absorb liquids. During the treatment phase of the study, animal room temperature and relative humidity were continuously recorded, using a seven day recorder. Minimum and maximum parameters were noted daily and ranged from 15 to 22.5°C and 36 to 78%, respectively. Air exchange was set to provide approximately 18 air changes per hour. Fluorescent lighting was controlled by means of a time switch and provided 12 hours of artificial light (0700 - 1900 hours) which was followed by 12 hours of darkness in each 24 hour period.

F. Methods:

1. Animals: The three female rabbits, nulliparous and nonpregnant, were allocated to the study using a random numbers table. The randomised list of cage numbers 1-50 (animal numbers 1414-1463) was generated using the statistical software package Genstat version 5 Release 3.2, utilising the randomisation directive (Payne R.W *et al* 1993*Genstat 5 Release 3 Reference Manual.* Clarendon Press Oxford). Animals were in the bodyweight range 2304 g to 2571 g and at least 12 weeks of age on Day 1 of the study. The animals were acclimatised to the laboratory environment as follows: Screen animal 15 days (8 October to 22 October with dosing on 23 October; additional two animals 27 days(8 October to 3 November with dosing on 4 November).

2. Dosing: Not exceeding one hour, prior to instillation of the test substance the eyes of each animal were examined to ensure that there was no pre-existing corneal damage, iridial or conjunctival inflammation. One animal was treated in advance of the others, to ensure that if a severe response was produced, no further animals would be exposed (pilot animal see Table 1). A single 0.1 ml dose of the test substance, was placed undiluted into the lower everted lid of one eye of each animal. The eyelids were gently held together for one second after instillation before releasing. The contralateral eye remained untreated.

3. Observations: The rabbits were observed twice daily for mortality and morbidity.

4. Body Weights: The rabbits were weighed on arrival, immediately prior to dosing and at sacrifice.

5. Clinical signs: The rabbits were observed daily for any signs of ill health and toxicity. Observations were made at the cageside during the twice daily standard mortality and morbidity checks and when animals were removed from the cage to determine ocular responses.

6. Ocular Responses: The eye of the rabbits was examined 1 hour (approximately 60 minutes) and 1, 2 and 3 days after instillation (approximately 24, 48 and 72 hours). Additional observations were made for all animals 4, 7, 14 and 21 days after instillation. Observation of the eyes was aided by the use of a handheld light. At each interval, ocular irritation was assessed according to the following prescribed arbitrary numerical system (based on Draize JH, Appraisal of the Safety of Chemicals in Foods, Drugs & Cosmetics, Assoc. Food & Drug Officials of the US, Austin, TX; 1959):

Cornea

(A) Opacity: degree of density (most dense area used)

No ulceration or opacity	0
Scattered or diffuse areas, details of iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	2*
Opalescent areas, no details of iris visible, size of pupil barely discernible	3*
Opaque, iris invisible	4*

(B) Area of cornea involved

One quarter (or less) but not zero	1
Greater than one quarter but less than half	2
Greater than half but less than three quarters	3
Greater than three quarters up to whole area	4

The Total Score = (A x B) x 5, Maximum Total = 80

(C) Iris

Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, haemorrhage, gross destruction (any or all of these)	2*

The Total Score = C x 5, Maximum Total = 10

Conjunctivae

(D) Redness (refers to the most severe reading of palpebral and bulbar conjunctivae, as compared to the control eye)

Blood vessels normal	0
Some blood vessels definitely hyperaemic (injected)	1
Diffuse, crimson colour, individual vessels not easily discernible	2*
Diffuse beefy red	3*

(E) Chemosis (lids and/or nictating membranes)

No swelling	0
Any swelling above normal (includes nictating membranes)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half-closed	3*
Swelling with lids more than half-closed	4*

(F) Discharge

No discharge	0
Any amount different than normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs and considerable area around the eye	3

The Total Score = (D + E + F) x 2, Maximum Total = 20

The maximum total score is the sum of all scores obtained from the cornea, iris and conjunctivae. Maximum total score possible = 110.

* indicates a positive effect.

Any other lesion not covered by this scoring system was described.

For each animal, mean scores for corneal opacity, iris lesions, conjunctival erythema and conjunctival chemosis were calculated by adding the scores for the respective parameter at 24, 48 and 72 hours and dividing by three. The irritation potential of the test substance was assessed according to the criteria described in Appendix 2 which are based upon the European Economic Community guidelines (93/21/EEC).

8. Animal Disposition: After the final observation (November 13, 1998 for screen animal or November 25, 1998 for the remaining two animals) the rabbits were sacrificed by an intravenous overdose, into the marginal ear vein, of pentobarbitone sodium B.P. 200 mg/ml (Euthatal manufactured by Rhône Mérieux Ltd., Harlow, Essex, England) and discarded without necropsy.

G. Location of Study Records: The protocol, protocol amendment and all raw data as well as a sample of the test substance and study related documents generated during the course of the study at Huntingdon Life Sciences Ltd., together with the original final report are lodged in the Huntingdon Life Sciences Ltd., Archive, Huntingdon, England. Such records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five year retention period the client will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the client's prior approval.

H. Statistical Analysis: None conducted.

III. RESULTS

A. Mortality: No deaths occurred during the study.

B. Body Weights: The weight for the rabbits used in this study was in the range 2304 and 2571 g at treatment initiation.

C. Clinical Signs: There were no signs of systemic reaction to treatment.

D. Ocular Responses: (Screen and Main Study): A single instillation of nto the eye of the rabbit elicited dulling of the cornea developing into corneal opacification (Grade 1) in one animal, corneal opacification (Grade 2 or 3) in two animals and vascularisation on the cornea in all three animals. Iridial inflammation, a diffuse beefy red colouration of the conjunctivae, swelling with lids about half-closed and discharge with moistening of the lids and hairs and considerable area around the eyes was also seen in all three animals. Corneal opacification (Grade 1) persisted in two animals at study termination 21 days

after instillation, together with hyperaemia of the blood vessels of the conjunctivae with or without slight swelling in all three animals.

IV. CONCLUSION

Under the conditions of this study, elicited corneal opacification with vascularisation, iridial inflammation and considerable conjunctival irritation and based on European Economic Community guidelines (93/21/EEC) is classified as a severe irritant.

V. DEVIATIONS FROM PROTOCOL

There were no deviations that were considered to have affected the quality or integrity of the data from the study. However the following deviations did occur:

During the study the lower and upper temperature values recorded were 15 and 22.5°C respectively. These values were outside the range of 17-21°C for temperature stated in the protocol.

The higher value for humidity recorded was 78%. This exceeded the 30 - 70% tolerance stated in the protocol.

Information regarding the pH of the test substance was not available from the Sponsor prior to the start of the study. Therefore in order to comply with regulatory and UK Home Office guidelines, the pharmacy department at Huntingdon Life Sciences measured the pH of the test substance, 10%, using a pH meter. The resultant measurement was used in conjunction with other practices to enhance animal welfare. However the Sponsor has indicated that based upon the nature of the test substance the measurement of the pH was inappropriate.

Contrary to Huntingdon Life Sciences protocol study number), the water from the automatic watering system exit was not sampled on this occasion. It is not considered that this omission had any effect on the scientific interpretation of the study.

There were no other deviations.

VI. TABLE 1

Ocular Reactions

Rabbit number and sex	Region of eye			One hour	Day after instillation							Result Positive (+) Negative (-)
					1	2	3	4	7	14	21	
1444 Female*	Cornea	A.	Density	0	0	1	1	2	1	1	1	+
		B.	Area	0	0	2	3	3	2	2a	2a	
	Total (AxB)x5			0	0	10	15	30	10	10	10	
	Iris	A.		0	1	1	1	1	0	0	0	
	Total Ax5			0	5	5	5	5	0	0	0	
	Conjunctiva	A.	Redness	3	3	2	2	2	2	1	1	
		B.	Chemosis	3	3	2	1	2	1	0	0	
		C.	Discharge	3	2	1	2	2	0	0	0	
	Total (A+B+C)x2			18	16	10	10	12	6	2	2	
	Total Score			18	21	25	30	47	16	12	12	
1427 Female	Cornea	A.	Density	D	1	1	1	1	1	1	1	+
		B.	Area	2	2	2	2	3	2	1a	1a	
	Total (AxB)x5			0	10	10	10	15	10	5	5	
	Iris	A.		0	1	1	1	1	0	0	0	
	Total Ax5			0	5	5	5	5	0	0	0	
	Conjunctiva	A.	Redness	3	3	2	2	2	1	1	1	
		B.	Chemosis	3	2	2	2	2	0	0	0	
		C.	Discharge	3	3	2	2	2	0	0	0	
	Total (A+B+C)x2			18	16	12	12	12	2	2	2	
	Total Score			18	31	27	27	32	12	7	7	
1457 Female	Cornea	A.	Density	0	1	1	1	1	3	2	0	+
		B.	Area	0	2	4	4	4	1	2a	0	
	Total (AxB)x5			0	10	20	20	20	15	20	0	
	Iris	A.		0	1	1	1	1	0	0	0	
	Total Ax5			0	5	5	5	5	0	0	0	
	Conjunctiva	A.	Redness	2	2	3	3	3	3	2	1	
		B.	Chemosis	3	2	2	2	2	2	1	1	
		C.	Discharge	3	2	2	2	2	0	0	0	
	Total (A+B+C)x2			16	12	14	14	14	10	6	4	
	Total score			16	27	39	39	39	25	26	4	

* Screen animal
a Vascularisation on the cornea
D Dulling of the cornea

VII. APPENDIX 1

Certificate of analysis for microbial contaminants of water

CERTIFICATE OF ANALYSIS

MICROBIOLOGICAL ANALYSIS OF ANIMAL DRINKING WATER

Huntingdon Life Sciences study number :		
Report number :		
Source of water sample (s):	Huntingdon Research Centre, Building R14 Room 2 (1) Cold water tap entry. (2) Automatic watering system exit*.	
Date sampled and tested :	30 September 1998	
Test procedure :	Protocol for Huntingdon Life Sciences study number study number approved 11 June 1998.	
Research Laboratory :	Huntingdon Research Centre Department of Cellular Sciences P O Box 2 Huntingdon Cambridgeshire PE18 6ES ENGLAND	
RESULTS	**Count**	**Specification**
Total viable count for aerobic bacteria :	(1) 1 cfu/ml (22°C)	$<10^4$ cfu/ml (22°C)
	(1) <1 cfu/ml (37°C)	$<10^2$ cfu/ml (37°C)
Total viable count for presumptive coliform bacteria :	(1) <1 cfu/100ml	<1 cfu/100ml
Total viable count for presumptive *E.coli* :	(1) <1 cfu/100ml	<1 cfu/100ml
CONCLUSION:	Sample (1) showed satisfactory microbiological quality.	
Protocol Deviations:	* Contrary to the protocol, the Automatic watering system exit was not sampled on this occasion.	
Results reviewed by : **Head, Microbiology**	**Signature :** **Date :** 13 May 1999	

cfu - colony forming unit

VII. APPENDIX 2

Criteria for assessment of the irritation potential of the test substance based on European Economic Community guidelines (93/21/EEC)

- The test substance will be considered an EEC irritant if two or more animals have a corneal opacity mean score greater than or equal to 2.0 but less than 3.0, an iris lesion mean score greater than or equal to 1.0 but less than 2.0, a conjunctival erythema mean score greater than or equal to 2.5, or a chemosis mean score greater than or equal to 2.0.

- The test substance will be considered a EEC severe irritant if either the corneal opacity mean score in two or more animals is greater than or equal to 3.0 or the iris lesion mean score is equal to 2.0. Ocular reactions are also severe when they are still present at the end of the observation time.

- If the above criteria is not met then the test compound may be considered to be a non-irritant.